

Western Blot of Flk-1/SEAPS Immunoprecipitation with MAb DC101

FIK-1 AP

# FIGURE 2A



# FIGURE 2B

- 97



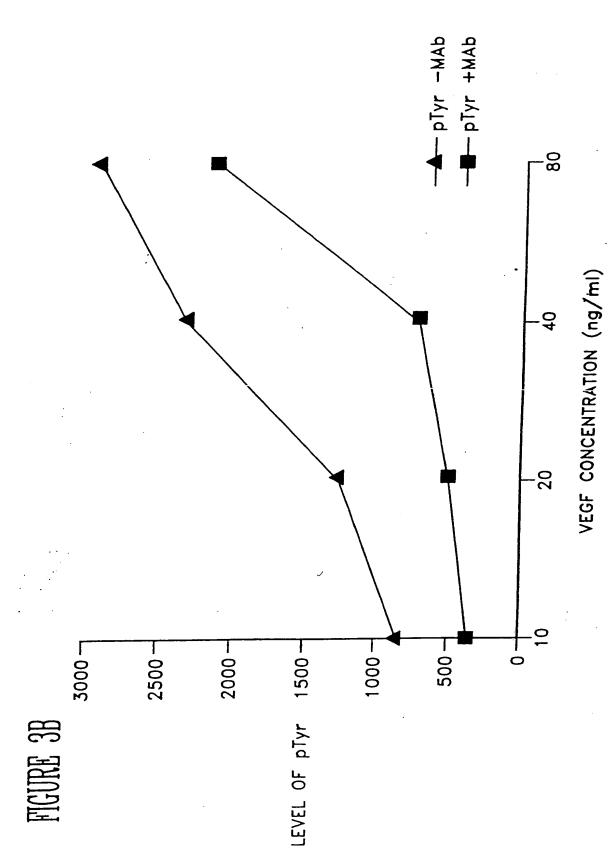
# FIGURE 3A

MAb (5μg/ml) VEGF (ng/ml)

M 100 11 M M M M M

- 200

**Anti-Ptyr** 





Inhibition of VEGF-Flk-1/fms activation by pre-bound MAb DC101

Assay conditions:

PC

MAb ( $5\mu g/ml$ ):

· 4 4

VEGF (ng/ml):

- - 20 40 40 40



Probe: Anti-Ptyr

Assay conditions: P: MAb pre-bound 15'; VEGF 15'

C: Competitive assay; MAb

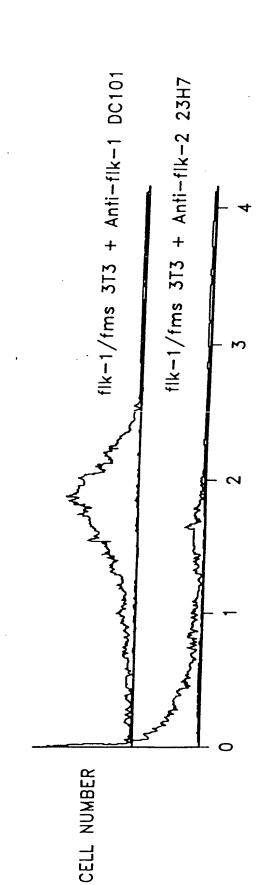
+ VEGF 15'



- 10 2.5 10 SE CM 20 40 20 40 40/ MAb (μg/ml) -VEGF<sub>165</sub> (ng/ml) -

- 200

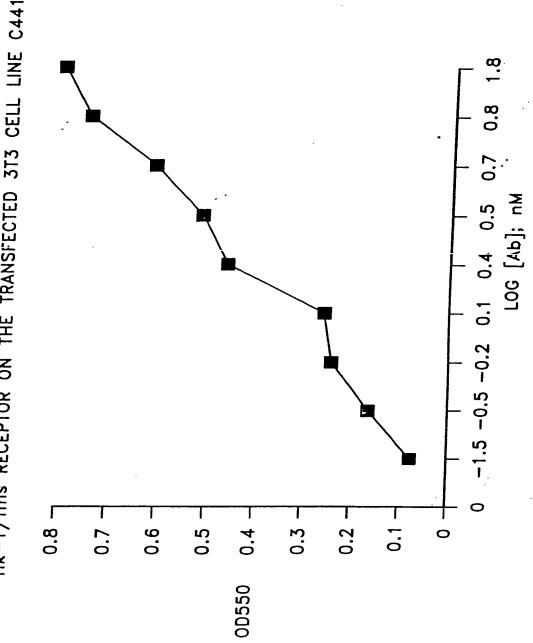
FIGURE 6 facs analysis of anti-fik-1 mad binding to fik-1/fms transfected 3T3 cells (C441)



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SATURATION BINDING OF MAB DC101 TO THE fik-1/fms RECEPTOR ON THE TRANSFECTED 3T3 CELL LINE C441





Immunoprecipitation of phosphorylated Flk-1/fms from VEGF stimulated Flk-1/fms transfected 3T3 cells

Antibody: 1 2 3 4

**200** 

Anti-pTyr

Antibodies:

- 1) Rat anti-flk-2 IgG<sub>2a</sub> 2A13
- 2) Rat anti-flk-1 IgG<sub>1</sub> DC101
- 3) Rat anti-flk-2 IgG<sub>1</sub> 23H7
- 4) Rabbit anti-fms polyclonal IM 133



Sensitivity of VEGF induced phosphorylation of the Flk-1/fms receptor to inhibition by MAb DC101

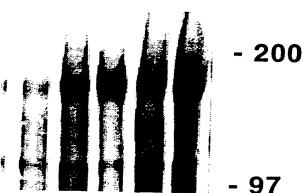
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Effect of MAb DC101 on CSF-1 induced phosphorylation of the fms receptor

MAb (5μg/ml) CSF-1 (ng/ml)

- + - - + + - - 20 40 20 40





Specificity of MAb DC101 neutralization of the activated Flk-1/fms receptor

Condition: VEGF ng/ml: MAb: (5μg/ml)

Competitive - 20 40 40 40 40 Prebound 40 40 40 2 3





- 200

Anti pTyr





#### **Anti-FMS**

Rat MAbs: 1) Anti-fik-1 IgG<sub>1</sub> DC101 2) Anti-fik-2 IgG<sub>1</sub> 23H7 3) Anti-fik-2 IgG<sub>2a</sub> 2A13

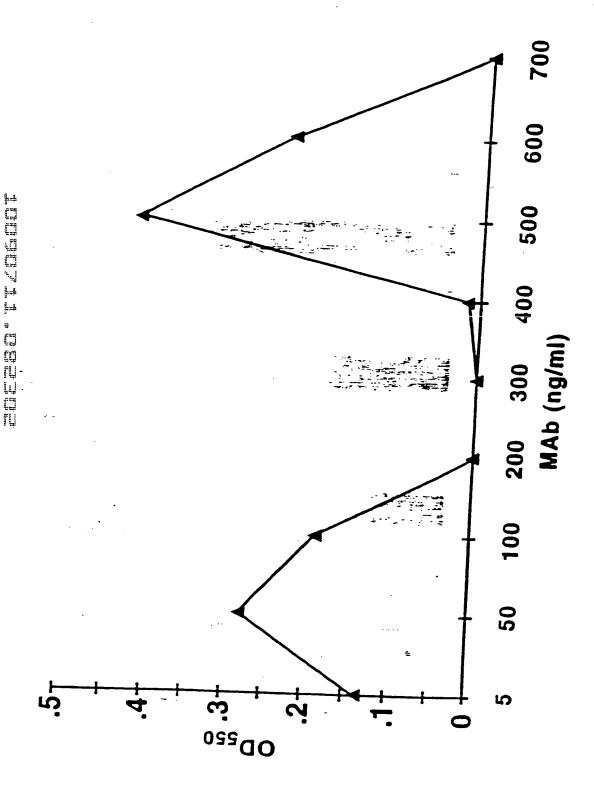


Figure 12

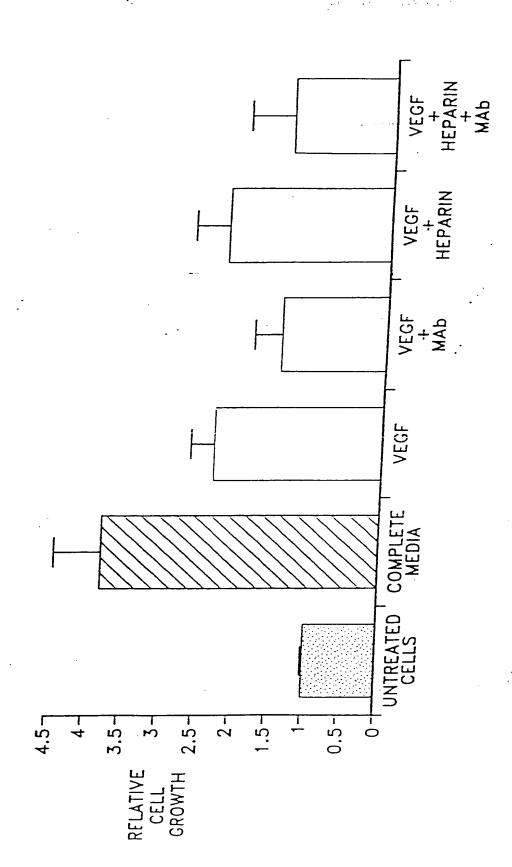


Immunoprecipitation of phosphorylated receptor bands from VEGF stimulated HUVEC cells

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# FIGURE 14







# Effect of MAb DC101 on VEGF Receptor Forms in the Tumor Cell Lines A431 and 8161

DC101 (5µg/ml): Cell Line: Heparin (1µg/mi): VEGF(20ng/ml):

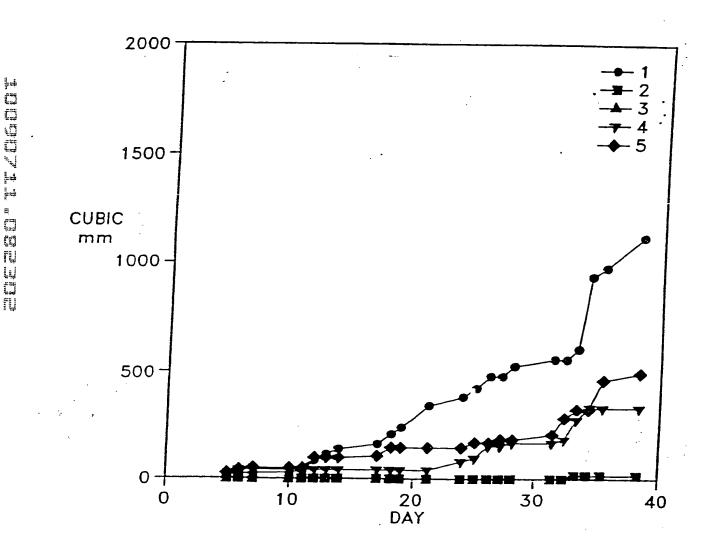
IP: MAb DC101 Probe: Anti-pTyr

Figure 15



#### FIGURE 16A

TREAMENT OF GLIOBLASTOMA
XENOGRAFTS WITH RAT anti-flk-1 MAb

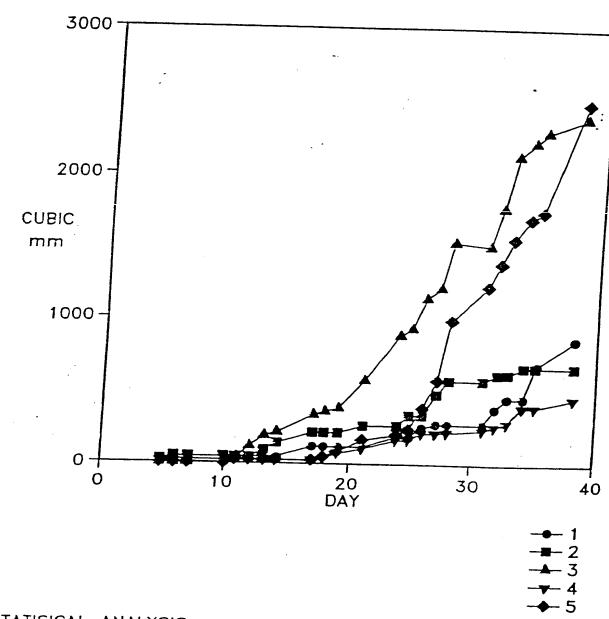


STATISICAL ANALYSIS flk-1 SLOPE = 16.09 p VALUE FOR flk-1 VERSUS flk-2 TUMOR SIZE = 0.0001

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#### FIGURE 16B

TREAMENT OF GLIOBLASTOMA XENOGRAFTS WITH RAT anti-flk-2 MAb



STATISICAL ANALYSIS
flk-2 SLOPE = 37.39
p VALUE FOR flk-1 VERSUS flk-2 TUMOR SIZE = 0.0001

